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THE UNITED STATES PATENT AND TRADEMARK OFFICE

Re: Appeal to the Board of Appeals

In re Application of: BORNSCHEUER et al.

Serial No. 09/161,680 Group Art Unit: 1652

Filed: September 28, 1998 Examiner: Kerr

For: ALTERATION OF THE SUBSTRATE SPECIFICITY OF ENZYMES

Date: August 3, 2004

To: Commissioner for Patents
Sir:

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2. [ ] A check to cover the extension fee of $___ is enclosed.
3. [X] BRIEF on appeal in this application is transmitted herewith.
4. [ ] An Oral Hearing is requested.
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Respectfully submitted,
KEIL & WEINKAUF

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re the application of	) **MAIL STOP APPEAL BRIEF**
	)
BORNSCHEUER et al.	) Group Art Unit: 1652
	)
Serial No. 09/161,680	) Examiner: Kerr
	)
Filed: September 28, 1998	)
	)

For: ALTERATION OF THE SUBSTRATE SPECIFICITY OF ENZYMES

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, PO Box 1450, Alexandria, VA 22313-1450, on:

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Honorable Comm'r. of Patents
PO Box 1450
Alexandria, VA 22313-1450

BRIEF ON APPEAL

Sir:

This appeal is from the examiner's more than the third time of the claims 12-27. The examiner's latest office action is dated March 3, 2004.

REAL PARTY IN INTEREST

The real party in interest is BASF Aktiengesellschaft, of Ludwigshafen, Germany. Reel/Frame 9536/0052, recorded on October 26, 1998.

RELATED APPEALS AND INTERFERENCES

To appellants' knowledge and belief, there are no interferences or other appeals which will directly affect or be directly affected by or have a bearing on the Board's decision in this application.

STATUS OF THE CLAIMS

Claims 12-27 currently are pending in the application. Claims 20 and 26 are rejected under 35 USC § 112, ¶2 as being indefinite. Claims 12-23 and 24-27 are rejected under 35 USC § 112, ¶1 as not complying with the written description requirement. Claims 12-27 are rejected under 35 USC § 112, ¶1 as not complying with the enablement requirement. Claims 24-27 are rejected under 35 USC § 112, ¶1 as presenting new matter.

STATUS OF THE AMENDMENTS

The last amendment of the claims was the reply under 37 CFR § 1.114 submitted on October 28, 2003.

SUMMARY OF THE INVENTION

The present invention relates to a method for the generation of new catalytic activities in enzymes. The synthetic chemist is frequently confronted with the problem of being unable to follow easy synthetic routes to prepare a chemical compounds because such routes would result in compounds in which, for example, the elimination of a required protective group is no longer possible since the synthesized molecule would be destroyed. Since enzymes cleave chemical bonds under mild conditions they can sometimes be used to solve synthetic problems in which a protective group can be eliminated under without destroying other bonds and thus the molecule. However, the enzymatic activity and/or stability is often inadequate for industrial use of the enzymes, so that although the chemical syntheses require a larger number of synthesis stages,

nevertheless they are less costly and are therefore implemented industrially.

Mutagenesis methods have been employed and are described in the prior art. However, these methods have the disadvantage that it is possible to optimize only enzymatic activities which are present. If new enzymatic reactions, i.e., new substrate specificities of the enzymes are required, for example, for cleaving a substrate, it is necessary first to search for this new enzymatic activity in an elaborate screening of natural forms. It therefore became an object of the present invention to develop a novel widely applicable method which does the have these disadvantages and which is able to generate new enzymatic activities rapidly and straightforwardly.

Applicants have found that this object is achieved by a method for generating new enzymatic activities which comprises carrying out the following steps: a) introducing a DNA sequence coding for the enzyme *Escherichia coli* strain XL1-Red or into a functional derivative thereof which is also an *E. coli* strain carrying the genetic markers relA1, mutS, mutT and mutD5 and having an increased mutation rate, b) incubating the transformed *Escherichia coli* strain XL1-Red or its functional derivative to generate mutations in the DNA sequence, c) transferring the mutated DNA sequence from the transformed *Escherichia coli* strain XL1-Red or its functional derivative to a microorganism which has no enzyme activity which would impeded selection, d) incubating this microorganism to detect the new catalytic activity in at least one selection medium which comprises at least one enzyme substrate to recognize the newly generated catalytic activity in the enzyme, with or without other indicator

substances, and e) selecting the microorganism which show the newly generated catalytic activity, said microorganisms in steps c), d) and e) being a member selected from the group consisting of bacteria, fungi and yeasts wherein the enzyme is selected from the group consisting of lipases, amidases, nitrilases, ether hydrolases, peroxidases, glycosidases and phytases.

ISSUES

Whether claims 20 and 26 are indefinite under 35 USC § 112, ¶2.

Whether claims 12-23 and 24-27 comply with the written description requirement under 35 USC § 112, ¶1.

Whether claims 12-27 comply with the enablement requirement 35 USC § 112, ¶1.

Whether claims 24-27 present new matter under 35 USC § 112, ¶1.

GROUPING OF CLAIMS

The claims have not been argued separately.

ARGUMENT

The following legal authorities are relied on in the following arguments in the order in which they are cited:

Orthokinetics, Inc. v. Safety Travel Chairs, Inc., 806 F.2d 1565, 1576, 1 USPQ2d 1081, 1088 (Fed. Cir. 1986)

Lockwood v. American Airlines, Inc., 41 USPQ2d 1961, 1966 (Fed. Cir. 1997)

Amgen, Inc. v. Chugai Pharmaceutical, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991)

In re Deuel, 34 USPQ2d 1210 (Fed. Cir. 1995)

Fujikawa v. Wattanasin, 39 USPQ2d 1895 (Fed. Cir. 1996)

In re Ruschig, 154 USPQ 118 (CCPA 1967)

THE OBJECTION AND REJECTIONS

Objection

The examiner objected to the specification for being confusing on pages 3-4 as amended on April 15, 2003 because it read "The generation of new catalytic activity reduced the K_m or increases the k_{kat} or both." The examiner had noted that "this is confusing because if no activity was present prior to mutation, from what value should the K_m or k_{kat} be measured as reduced or increased?"

Applicants believe that a reference value for K_m and/or k_{kat} is unnecessary for present purposes. In the present invention, the relative change is to be observed qualitatively, and the numerical value of the change is not important. Whether such change is due specifically to a reduction in K_m or an increase in k_{kat} and to what precise extent it is due to either, is of little or no import.

Rejection of claims 20 and 26 under 35 USC § 112, ¶2

Claims 20 and 26 are rejected under 35 USC § 112, ¶2 as being indefinite because the nature of the enzymes in the list is unclear.

The amendments to Table I on page 10 of the specification introduced the trade

names of the commercially available enzymes employed in the examples beginning on page 9. Applicants submitted printed pages from various sources which indicate that the terms Amano, Lipoxyme, and Novozyme (amended herein to "Novozym," which is technically more correct) would be inherently observed by one of skill in the art when reading the information in the table as originally filed.

Applicants also attached pages from the Amano Enzyme, Inc. website, which list the following products: Lipase PS "Amano," Lipase AH "Amano," Acylase "Amano," Lipase D "Amano," Lipase F-AP 15, Lipase AY "Amano," Lipase M "Amano" 10, Lipase R "Amano," and Lipase G "Amano" 50. Pages taken from the Sanger Institute website and from the German Collection of Microorganisms and Cell Cultures (DSMZ) website, also were attached, and they indicate the equivalence between *Burkholderia cenocepacia* and *Pseudomonas cepacia*, and *Rhizopus oryzae* and strains *R. javanicus* and *R. delamar*, respectively.

Pages printed from the Novozymes A/S website indicate use of the words "Novozym" and "Lipozyme" for commercially available products manufactured by that company. The abstract of Yamamoto, et al. recite use of "two lipases[:] Amano PS (*Pseudomonas* sp.) and Novozym 435®," indicating recognized use of these terms by skilled artisans. Likewise, the paper by Maugard and Legroy indicates that "Novozym® SP 435 (lipase from *Candida antarctica* immobilised on an acrylic resin), [and] Lipozyme® (lipase from *Rhizomucor miehei* immobilised on an anionic macroporous resin ...), were [obtained] from Novo Industries (Denmark)." Novo has since moved its

industrial enzyme production to Novozymes A/S (*see* printed page from Novozymes referencing the Demerger Document).

As indicated above, Lipase PS and Lipase AH are art-recognized portions of trade names under which certain lipases from *Pseudomonas candida* are sold by Amano Enzyme, Inc. The name "Amano" is trademarked in the United States, and as such, should not be used in the claims. The letters PS and AH serve to indicate particular lipase preparations are known in the art. From this, and from the above discussion of the relevant literature, what is meant by "*Pseudomonas cepacia* lipase PS" and "*Pseudomonas cepacia* lipase AH" would be abundantly clear to the skilled artisan. The test for definiteness under 35 USC § 112, ¶2, is whether "those skilled in the art would understand what is claimed when the claim is read in light of the specification." *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1576, 1 USPQ2d 1081, 1088 (Fed. Cir. 1986).

Rejection of claims 12-27 under 35 USC § 112, ¶1, written description

The examiner has rejected claims 12-23 and 24-27 under 35 USC § 112, ¶1, because the examiner believes the present claims are drawn to using any enzyme and any new substrate to produce a new enzyme with altered substrate specificity relative to the original. The examiner stated that the specification provides a single example of such enzymes and substrates and no correlations between their structures and functions. The examiner believes that the written description of a single example in the instant specification does not adequately describe the genus of "reagents" claimed for

use in the methods.

The examiner stated that a "representative number" of species is required to adequately describe the full scope of functional equivalents. However, "possession" of the invention need not be shown by any actual reduction to practice (*see, e.g., Lockwood v. American Airlines, Inc.*, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997)). Rather, a compound must be defined by "whatever characteristics sufficiently distinguish it" (*Amgen, Inc. v. Chugai Pharmaceutical*, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991)). There is no absolute requirement for description of an actual reduction to practice, so long as one of skill in the art would recognize that the disclosed element is obvious as disclosed, or may immediately envisage the element, relying on knowledge and level of skill in the art (*see, e.g., In re Deuel*, 34 USPQ2d 1210 (Fed. Cir. 1995); *Fujikawa v. Wattanasin*, 39 USPQ2d 1895 (Fed. Cir. 1996); *In re Ruschig*, 154 USPQ 118 (CCPA 1967)).

In the present case, the specification and claims indicate that functional equivalents of the present mutator strain are derivatives of *Escherichia coli* XL1 Red which possess the gene markers relA1, mutS, mutT and mutD5. Production of such derivatives of *E. coli* XL1 Red, given the knowledge and level of skill in the art, would be obvious and straightforward to one of ordinary skill therein. It is respectfully submitted that the present disclosure, coupled with the extensive resources available to the practitioner of genetic recombination, are sufficient to support the claimed range of functional derivatives for purposes of the written description requirement.

Rejection of claims 12-27 under 35 USC § 112, ¶1, enablement

The examiner rejected claims 12-23 and 24-27 under the enablement requirement because the specification, while being enabling for specific examples of the methods proven to achieve their goals, does not reasonably provide enablement for methods using all enzymes, all substrates and all possible mutator strains.

Applicants believe the nature of the experimentation required for claims 12-23, though potentially arduous and substantial, would be a routine matter for the skilled artisan. One of skill in the art would recognize how divergent from an enzyme's original substrate a particular substrate may be to ensure that the new catalytic activity can be produced in that enzyme. The field of enzymology is not so unpredictable that one of ordinary skill in the art would be unable to understand the necessary parameters inherent in practicing the present invention. To answer the examiner's questions, one of skill in the art would apply the knowledge and understanding commonly held with regard to the individual enzyme to be mutated and/or substrate targeted.

Rejection of claims 12-27 under 35 USC § 112, ¶1, new matter

The examiner states that the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The examiner believes the concept of using this method to generate a "new catalytic activity... within the same International Union of Biochemistry class as the enzyme's original activity" is not found in the specification as originally filed.

Claims 24-27 are limited to the process wherein the new catalytic activity is within that IUB class already assigned to the original enzyme. Support for this limitation is found in the specification at page 4, line 10, which introduces the IUB classification system, and from the example beginning on page 11, in which the new catalytic activity is within the IUB class assigned to the enzyme originally.

CONCLUSION

For the foregoing reasons, it is respectfully submitted that reversal of the examiner's rejection of all claims is in order.

Please charge any shortage in fees due in connection with the filing of this paper, including Extension of Time fees to Deposit Account No. 11-0345. Please credit any excess fees to such deposit account.

Respectfully submitted,

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APPENDIX

12. A method for generating a new catalytic activity in an enzyme, comprising the steps of:

a) introducing a DNA sequence coding for the enzyme into the *Escherichia coli* strain XL1-Red or into a functional derivative thereof which is also an *E. coli* strain carrying the genetic markers relA1, mutS, mutT and mutD5 and having an increased mutation rate,

b) incubating the transformed *Escherichia coli* strain XL1-Red or its functional derivative to generate mutations in the DNA sequence,

c) transferring the mutated DNA sequence from the transformed *Escherichia coli* strain XL1-Red or its functional derivative to a microorganism which has no enzyme activity which would impede selection,

d) incubating this microorganism to detect the new catalytic activity in at least one selection medium which comprises at least one enzyme substrate to recognize the newly generated catalytic activity in the enzyme, with or without other indicator substances, and

e) selecting the microorganisms which show the newly generated catalytic activity, said microorganisms in steps c), d) and e) being a member selected from the group consisting of bacteria, fungi and yeasts,

wherein the enzyme is selected from the group consisting of lipases, amidases, nitrilases, ether hydrolases, peroxidases, glycosidases and phytases.

13. The method of claim 12, wherein the enzyme is a lipase.

14. The method of claim 12, wherein the enzyme is an amidase.

15. The method of claim 12, wherein the enzyme is a nitrilase.

16. The method of claim 12, wherein the enzyme is an ether hydrolase.

17. The method of claim 12, wherein the enzyme is a peroxidase.

18. The method of claim 12, wherein the enzyme is a glycosidase.

19. The method of claim 12, wherein the enzyme is a phytase.

20. The method of claim 13, wherein the lipase is selected from the group of lipases consisting of *Pseudomonas cepacia* lipase PS, *Pseudomonas cepacia* lipase AH, acylase, *Rhizopus delamar* lipase, *Rhizopus javanicus* lipase, *Candida rugosa* lipase, *Mucor javanicus* lipase, *Penicillium roquefortii* lipase, *Penicillium cyclopium* lipase, *Chromobacterium viscosum* lipase, *Rhizomucor miehei* lipase, *Humicola lanuginosa* lipase, *Candida antarctica* lipase B and *Candida antarctica* lipase A.

21. The method of claim 12, wherein steps (a) to (e) are performed several times in sequence by reisolating and retransforming the DNA sequence from the microorganisms selected in step (e) to the strain *Escherichia coli* XL-1 Red or its functional derivative.

22. A method for generating a new catalytic activity in an enzyme, comprising the steps of:

a) introducing a DNA sequence coding for the enzyme into the *Escherichia coli* strain XL1-Red or into a functional derivative thereof which is also an *E. coli* strain carrying the genetic markers relA1, mutS, mutT and mutD5 and having an increased mutation rate,

b) incubating the transformed *Escherichia coli* strain XL1-Red or its functional derivative to generate mutations in the DNA sequence,

c) transferring the mutated DNA sequence from the transformed *Escherichia coli* strain XL1-Red or its functional derivative to a microorganism which has no enzyme activity which would impede selection,

d) incubating this microorganism to detect the new catalytic activity in at least one selection medium which comprises at least one enzyme substrate to recognize the newly generated catalytic activity in the enzyme, with or without other indicator substances, and

e) selecting the microorganisms which show the newly generated catalytic

activity, said microorganisms in steps c), d) and e) being a member selected from the group consisting of bacteria, fungi and yeasts,

wherein the enzyme is an esterase selected from the group consisting of *Pseudomonas fluorescens* esterase, pig liver esterase and *Thermoanaerobium brockii* esterase.

23. The method of claim 22, wherein steps (a) to (e) are performed several times in sequence by reisolating and retransforming the DNA sequence from the microorganisms selected in step (e) to the strain *Escherichia coli* XL-1 Red or its functional derivative.

24. A method for generating a new catalytic activity in an enzyme, wherein the new catalytic activity is within the same International Union of Biochemistry class as the enzyme's original catalytic activity, comprising the steps of:

a) introducing a DNA sequence coding for the enzyme into the *Escherichia coli* strain XL1-Red, or into a functional derivative thereof which is also an *E. coli* strain carrying the genetic markers relA1, mutS, mutT, and mutD5, and having an increased mutation rate,

b) incubating the transformed *Escherichia coli* strain XL1-Red or its functional derivative to generate mutations in the DNA sequence,

c) transferring the mutated DNA sequence from the transformed *Escherichia coli*

strain XL1-Red or its functional derivative to a microorganism which has no enzyme activity which would impede selection,

d) incubating this microorganism to detect the new catalytic activity in at least one selection medium which comprises at least one enzyme substrate to recognize the newly generated catalytic activity, with or without other indicator substances, and

e) selecting the microorganisms which show the newly generated catalytic activity, said microorganisms in steps c), d) and e) being a member selected from the group consisting of bacteria, fungi and yeasts, wherein the enzyme is selected from the group consisting of lipases, amidases, nitrilases, ether hydrolases, peroxidases, glycosidases, phytases, and esterases selected from the group consisting of *Pseudomonas fluorescens* esterase, pig liver esterase and *Thermoanaerobium brockii* esterase.

25. The method of claim 24, wherein the enzyme is a lipase.

26. The method of claim 25, wherein the lipase is selected from the group of lipases consisting of *Pseudomonas cepacia* lipase PS, *Pseudomonas cepacia* lipase AH, acylase, *Rhizopus delamar* lipase, *Rhizopus javanicus* lipase, *Candida rugosa* lipase, *Mucor javanicus* lipase, *Penicillium roquefortii* lipase, *Penicillium*

cyclopium lipase, *Chromobacterium viscosum* lipase, *Rhizomucor miehei* lipase, *Humicola lanuginosa* lipase, *Candida antarctica* lipase B and *Candida antarctica* lipase A.

27. The method of claim 24, wherein steps (a) to (e) are performed several times in sequence by reisolating and retransforming the DNA sequence from the microorganisms selected in step (e) to the strain *Escherichia coli* XL-1 Red or its functional derivative.